REDUCING ACTION OF TRYPANOSOMES ON HAEMOGLOBIN

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Amongst the blood changes occurring in animals infected with trypanosomes there is one which, so far as we can ascertain, has escaped observation. Whilst performing experiments with the object of investigating the cause of auto-agglutination of red blood cells*—a phenomenon which is frequently to be observed in cover-slip preparations of the fresh blood of infected animals—it was observed by one of us (W.Y.) that the colour of the erythrocytes in certain of the capillary tubes had changed from the normal red tint to a deep purple.

Further examination showed that this alteration in colour only occurred when the plasma, which had been added to the suspension of washed erythrocytes in normal saline solution, contained a considerable number of actively motile trypanosomes. The appearance was most striking in the tests carried out at 37° C. It was not so distinct in the tubes kept at 15° C., and was absent from those which had been placed in the ice-chest.

The fresh blood of a large number of animals of different species in various stages of infection was examined as regards its colour.

Technique.—A few drops of blood were allowed to fall from a vein of the ear into a tube containing a small amount of citrated saline solution. The red blood cells were then quickly thrown down by centrifugalisation and their colour noted. As a rule the

* Yorke. 'Auto-agglutination of Red Blood Cells in Trypanosomiasis,' Roy. Soc. Proc., 1910, Vol. LXXXIII, p. 238.

purple colour, when present, could easily be seen as the blood escaped from the wounded vein.

Only comparatively rarely were the red blood cells found to exhibit a markedly purple colour. It was occasionally seen in infected rats when the parasites in the peripheral blood were exceedingly numerous. As a rule, however, the blood of these animals was found to present the normal red colour even when it was swarming with trypanosomes.

Durham,* in a recent paper, has drawn attention to this fact. He states that, in rats suffering from Nagana, the blood in an advanced stage of the disease is sometimes of a dull purplish or chocolate colour. Examination with the spectroscope showed the bands of oxyhaemoglobin.

The blood of some of our infected rabbits exhibited this change of colour in a marked degree during the later stages of the disease. In the case of a rabbit infected with *T. rhodesiense*, the blood, as it escaped from the incised vein, was of an exceedingly purple colour.

The blood of this animal was subjected to various tests with a view to obtaining information as to the cause of this appearance.

In the first place it was found that when the blood was thoroughly shaken with air, or, when air was blown through it, the dull purple tint gradually gave place to the bright red colour of normal oxyhaemoglobin, until, finally, the appearance was identical with that of normal blood. The conversion of the purple colour into the red was not particularly rapid, and the blood required to be intimately mixed with air before it was accomplished. The fact that the red blood cells could be washed many times in large volumes of normal salt solution and still retain a distinctly purple colour illustrates how stable is the condition.

It will be noted that this observation is at variance with the results obtained by Durham with the blood of rats infected with *T. brucei*. Durham states that the dull coloured blood of these animals may be shaken with air, or allowed to stand for a week or more without developing the full red of normal oxyhaemoglobin.

Spectroscopic examination of a solution made by adding a few

* 'Notes on Nagana and some Haematozoa observed during my travels,' Parasitology, 1908, p. 227.

drops of the blood of this rabbit to distilled water revealed the ordinary bands of oxyhaemoglobin. No abnormal absorption bands were seen. When, however, the blood was added to previously boiled distilled water, avoiding as far as possible contact with air, the spectroscopic appearances were very different. Well-marked bands of oxyhaemoglobin were visible as before, but the space between the bands was also considerably darkened and the zone of absorption extended further towards the red. In other words, it was obvious that instead of the spectrum of a pure solution of oxyhaemoglobin we were dealing with that of a solution of partially reduced haemoglobin.

The dull purple colour of the blood is due, therefore, to the presence of a certain proportion of haemoglobin in the reduced form.

Some years ago Ehrlich demonstrated the ability of living cells to decolourise solutions of methylene blue.

Later it was shown by Klett,* Neisser and Wechsberg† and others that living leucocytes, spermatozoa, pancreas and kidney cells, and various micro-organisms all possessed this property. Neisser and Wechsberg found that these cells and bacteria lost their reducing capacity after they had been treated with toxic substances.

Ricketts‡ observed that emulsion of nervous tissue caused reduction of a solution of methylene blue. Further investigation indicated that intact cells were not essential for this reduction, and that emulsions which had been kept in the ice-chest for a week still reduced, although less vigorously than when fresh.

Experiments were performed by us with the object of ascertaining whether actively motile trypanosomes had a reducing action on solutions of methylene blue.

Solutions of various concentration were prepared by dissolving the dye in water to which sufficient sodium chloride had been added to make it isotonic. Small cells of a capacity of approximately 1 c.c. and 10 mm. in depth were completely filled with a mixture

* 'Zur Kenntniss der reducirenden Eigenschaften der Bakterien,' Zeit. für Hygiene, 1900, S.137.

† Ueber eine neue einfache Methode zur Beobachtung von schädigungen lebender Zellen und Organismen (Bioskopie),' Münch. med. Woch., 1900, S.126.

‡ 'Reduction of Methylene Blue by Nervous Tissue,' Journal of Infectious Diseases, 1904, Vol. I, p. 590.

consisting of equal portions of citrated plasma containing many actively motile trypanosomes in suspension and the isotonic solution of methylene blue. The cells were then sealed with cover-slips and placed in the incubator at 37° C.

It was found that in the course of a few minutes the fluid in the cells containing numerous trypanosomes and only weak solutions of methylene blue (0·05 per cent., or less) had completely lost its bluish tint and become colourless, indicating that complete reduction of the dye had resulted. On the other hand, no reduction was observed in the cells which contained the more concentrated solutions of methylene blue. It was found that solutions of the dye equal to 0·5 per cent. speedily caused the parasites to become motionless and die.

In view of the injurious effect of solutions of methylene blue on the parasites we were obliged to seek another indicator, the presence of which did not prevent the trypanosomes from carrying out their physiological functions.

Ultimately, it was decided to use isotonic solutions of rabbit haemoglobin. Solutions of this substance were found to possess many advantages.

(1) It had no injurious action on the parasites.

(2) The strength of the solution could be easily measured, and we were thus enabled to perform experiments of a quantitative character.

(3) An obvious change of colour occurs when such solutions are undergoing reduction.

(4) By the aid of the spectroscope it is possible to determine when reduction is complete.

Technique.—Blood from a vein of the ear of a rabbit was allowed to drop into citrated saline solution. The red blood cells were then separated from the citrated plasma by centrifugalisation, and subsequently washed several times with physiological salt solution. The washed erythrocytes were then laked by the addition of distilled water, and after the lapse of a few minutes sufficient sodium chloride was added to render the solution isotonic. A light precipitate, consisting for the most part of red cell stromata, appeared upon the addition of the salt and was thrown

down by means of the centrifuge and the clear solution of haemoglobin withdrawn.

The haemoglobin content of the solution in terms of human wet red cells was then determined by means of a haemoglobinometer reading.*

The suspension of trypanosomes was obtained by adding four volumes of the blood of an infected animal to one volume of a solution containing 1 per cent. sodium citrate and 0·9 per cent. sodium chloride. The red corpuscles having been thrown down by the aid of the centrifuge, the citrated plasma containing the trypanosomes in suspension was decanted off. The number of parasites present was determined by suitably diluting a small portion with sodium chloride solution and counting by means of a Thoma Zeiss haemocytometer.

A spectroscope tube of known capacity and 10 mm. in height was then completely filled with a mixture consisting of equal parts of the haemoglobin solution of known strength and of the suspension of trypanosomes containing a definite number of parasites per cubic millimetre. The tube was then sealed with a cover-slip and placed in the incubator at 37° C.

Control experiments were always made with the same solution of haemoglobin and the plasma of a normal animal of the same species diluted to a similar degree as the infected plasma.

After the expiration of only a few minutes a distinct change in colour was observed in the tubes which contained the suspension of trypanosomes. The bright red of the oxyhaemoglobin was being replaced by the dull purple colour of reduced haemoglobin. This process continued, until, finally, the colour became dark purple.

From time to time the tubes were examined spectroscopically by means of a Zeiss comparison spectroscope and the absorption bands compared with those of the standard solution of oxyhaemoglobin.

Since the contents of all the tubes gave absorption bands practically identical in appearance at the beginning of the experiment it was easily determined in which of the tubes changes

* Barratt and Yorke. 'An Investigation into the Mechanism of Production of Blackwater,' Annals of Tropical Medicine and Parasitology, 1909, Vol. III, p. 14.

were occurring by comparison with the standard solution which was kept in contact with the air at 15° C.

As the colour of the solutions became more and more purple the oxyhaemoglobin bands became less well defined. The space between the bands at D and E became darker, whilst the zone of absorption embracing the D line appeared to extend further towards the red. Finally, in place of the two distinct bands of oxyhaemoglobin there was only the single band of reduced hæmoglobin.

As will be seen from Table 1 the rapidity and degree of reduction varied, as a rule, directly with the number of parasites present in the citrated plasma. There were, however, certain well-marked exceptions. In Experiment 12, where the citrated plasma was derived from a rat heavily infected with *T. equiperdum*, there was scarcely any reduction of the haemoglobin solution, in spite of the fact that the infected plasma solution contained numerous trypanosomes (300,000 per c.mm.). When cover-slip preparations of the blood of this rat were examined it was found that the parasites were sluggishly motile and quickly clumped together into large masses and became motionless. Other analogous observations indicate that for appreciable reduction to occur it is essential for the trypanosomes to be actively motile. Trypanosomes killed by heating for a short time to 50° C. caused no reduction of methylene blue or haemoglobin solutions.

Having determined that actively motile trypanosomes exert a marked reducing action upon haemoglobin, we decided to continue our investigations with a view to ascertaining so far as possible in what manner the gaseous contents of the blood are altered by the action of living trypanosomes.

With this object in view, an analysis was made of the gases contained in a definite volume of defibrinated rabbit blood which had been treated for one hour at 37° C. with a certain amount of citrated plasma, containing numerous living trypanosomes. In the control experiments a similar volume of the same defibrinated blood was treated for a like time with an equal quantity of citrated plasma from a normal animal of the same species as the animal yielding the infected plasma. As a further control the gases present in the defibrinated blood alone were determined.

Table 1. Reducing action of trypanosomes on haemoglobin.

No. of experiment	Variety of trypanosomes	Equal Portions of Infected Plasma and Haemoglobin Solution		Time in which complete reduction occurred
		Number of parasites per cubic millimetre	Strength of haemoglobin solution	
1	*T. gambiense* ...	10,000	0·73 %	Complete in 1 hour
2	,, ...	18,250	0·75 %	Partial in 15 minutes
3	,, ...	30,000	0·86 %	Complete in 1 hour
4	,, ...	36,500	0·75 %	Complete in 10 minutes
5	,, ...	600,000	0·72 %	,,
6	*T. brucei*	300	0·75 %	Nil in 1 hour
7	,, ...	32,000	1·4 %	,,
8	,, ...	40,000	0·75 %	Complete in 10 minutes
9	*T. evansi*	3,000	1·0 %	Nil in 1 hour
10	,, ...	12,000	0·74 %	Complete in 15 minutes
11	*T. equiperdum* ...	100,000	0·72 %	,,
12	,, ...	300,000	1·00 %	Nil in 1 hour
13	*T. equinum*	100,000	3·4 %	Complete in 20 minutes
14	,, ...	170,000	3·4 %	Complete in 15 minutes

N.B.—In the control experiments where normal plasma was used instead of that of infected animals no reduction occurred.

TABLE 2. Analysis of gases obtained from defibrinated blood and from mixtures of this with plasma of normal and infected animals after incubation at 37° C. for 1 hour in the absence of air.

No. of experiment		Composition of Blood Mixture		Analysis of Gases obtained from Blood Mixture after Heating for 1 hour at 37° C. in the Absence of Air					
		Amount of defibrinated blood of normal rabbit	Amount of citrated plasma of normal or infected animal consisting of 2 parts plasma and 1 part citrated saline solution	Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
1	A	3 c.c.	—	0·730 c.c.	0·40 c.c.	0·28 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from normal rat	1·00 c.c.	0·60 c.c.	0·275 c.c.	0·125 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. equiperdum* containing 640,000 trypanosomes per c.mm.	0·745 c.c.	0·645 c.c.	0·025 c.c.	0·075 c.c.	0·25 c.c.	0·045 c.c.
2	A	3 c.c.	—	0·775 c.c.	0·390 c.c.	0·33 c.c.	0·055 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	0·97 c.c.	0·57 c.c.	0·29 c.c.	0·11 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 340,000 trypanosomes per c.mm.	0·76 c.c.	0·67 c.c.	0·007 c.c.	0·08 c.c.	0·283 c.c.	0·1 c.c.
3	A	3 c.c.	—	0·64 c.c.	0·34 c.c.	0·26 c.c.	0·04 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·14 c.c.	0·76 c.c.	0·315 c.c.	0·075 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei* containing 450,000 trypanosomes per c.mm.	0·65 c.c.	0·58 c.c.	0·00 c.c.	0·07 c.c.	0·315 c.c.	−0·18 c.c.
4	A	3 c.c.	—	0·67 c.c.	0·305 c.c.	0·31 c.c.	0·055 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	0·91 c.c.	0·535 c.c.	0·305 c.c.	0·07 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 580,000 trypanosomes per c.mm.	0·815 c.c.	0·64 c.c.	0·05 c.c.	0·125 c.c.	0·255 c.c.	0·105 c.c.
5	A	3 c.c.	—	0·935 c.c.	0·62 c.c.	0·275 c.c.	0·04 c.c.	—	—
	B	,,	1·5 c.c. from normal rat	1·11 c.c.	0·755 c.c.	0·255 c.c.	0·07 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. brucei*, containing 270,000 trypanosomes per c.mm.	0·73 c.c.	0·645 c.c.	0·03 c.c.	0·055 c.c.	0·235 c.c.	−0·11 c.c.
6	A	3 c.c.	—	1·09 c.c	0·715 c.c	0·315 c.c.	0·06 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·355 c.c.	0·96 c.c.	0·32 c.c.	0·075 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 800,000 trypanosomes per c.mm.	1·12 c.c	0·95 c.c.	0·09 c.c.	0·08 c.c.	0·23 c.c.	−0·01 c.c.

Table 2—*continued.*

No. of experiment		Composition of Blood Mixture		Analysis of Gases obtained from Blood Mixture after Heating for 1 hour at 37° C. in the Absence of Air					
		Amount of defibrinated blood of normal rabbit	Amount of citrated plasma of normal or infected animal consisting of 2 parts plasma and 1 part citrated saline solution	Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
7	A	3 c.c.	—	1·205 c.c.	0·755 c.c.	0·385 c.c.	0·065 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·38 c.c.	0·955 c.c.	0·32 c.c.	0·105 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 350,000 trypanosomes per c.mm.	1·11 c.c.	1·025 c.c.	0·01 c.c.	0·075 c.c.	0·31 c.c.	0·06 c.c.
8	A	3 c.c.	—	1·255 c.c.	0·82 c.c.	0·375 c.c.	0·06 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·505 c.c.	1·08 c.c.	0·345 c.c.	0·08 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 45,000 trypanosomes per c.mm.	1·19 c.c.	1·085 c.c.	0·025 c.c.	0·08 c.c.	0·32 c.c.	0·005 c.c.
9	A	3 c.c.	—	1·325 c.c.	0·865 c.c.	0·36 c.c.	0·10 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·68 c.c.	1·225 c.c.	0·37 c.c.	0·85 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 395,000 trypanosomes per c.mm.	1·23 c.c.	1·14 c.c.	0·05 c.c.	0·85 c.c.	0·32 c.c.	0·065 c.c.
10	A	3 c.c.	—	0·90 c.c.	0·42 c.c.	0·43 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from normal rat.	1·40 c.c.	0·86 c.c.	0·45 c.c.	0·09 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. dimorphon*, containing 250,000 trypanosomes per c.mm.	1·32 c.c.	1·015 c.c.	0·22 c.c.	0·85 c.c.	0·23 c.c.	0·065 c.c.
11	A	3 c.c.	—	0·785 c.c.	0·355 c.c.	0·38 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from normal rat.	1·105 c.c.	0·62 c.c.	0·39 c.c.	0·095 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. evansi*, containing 250,000 trypanosomes per c.mm.	0·875 c.c.	0·71 c.c.	0·08 c.c.	0·085 c.c.	0·31 c.c.	0·09 c.c.

TABLE 3. Data given in Table 2 re-calculated in volumes per cent.

No. of experiment		100 c.c. of mixture consisting of 2 parts defibrinated blood of normal rabbit and 1 part of citrated plasma from the following normal or infected animals	Analysis of Gases obtained from Blood Mixture after Heating in the absence of Air for 1 Hour at 37° C.					
			Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of CO_2 in C as compared with B
1	B	Normal rat	22·2 c.c.	13·3 c.c.	6·1 c.c.	2·8 c.c.	5·5 c.c.	1·0 c.c.
	C	Rat infected with *T. equiperdum*. The citrated plasma containing 640,000 trypanosomes per c.mm.	16·6 c.c.	14·3 c.c.	0·6 c.c.	1·6 c.c.		
2	B	Normal guinea-pig	21·6 c.c.	12·7 c.c.	6·4 c.c.	2·4 c.c.	6·25 c.c.	2·3 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 340,000 trypanosomes per c.mm.	17·0 c.c.	15·0 c.c.	0·15 c.c.	1·8 c.c.		
3	B	Normal guinea-pig	25·3 c.c.	16·9 c.c.	7·0 c.c.	1·7 c.c.	7·0 c.c.	−4·0 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 450,000 trypanosomes per c.mm.	14·4 c.c.	12·9 c.c.	0·0 c.c.	1·6 c.c.		
4	B	Normal guinea-pig	20·2 c.c.	11·9 c.c.	6·8 c.c.	1·6 c.c.	5·7 c.c.	2·3 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 580,000 trypanosomes per c.mm.	18·1 c.c.	14·2 c.c.	1·1 c.c.	2·8 c.c.		
5	B	Normal rat	24·7 c.c.	16·9 c.c.	5·8 c.c.	1·7 c.c.	5·1 c.c.	−2·6 c.c.
	C	Rat infected with *T. brucei*. The citrated plasma containing 270,000 trypanosomes per c.mm.	16·2 c.c.	14·3 c.c.	0·7 c.c.	1·2 c.c.		
6	B	Normal guinea-pig	30·1 c.c.	21·3 c.c.	7·1 c.c.	1·7 c.c.	5·1 c.c.	−0·2 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 800,000 trypanosomes per c.mm.	24·9 c.c.	21·1 c.c.	2·0 c.c.	1·8 c.c.		
7	B	Normal guinea-pig	30·7 c.c.	21·2 c.c.	7·1 c.c.	2·3 c.c.	6·9 c.c.	1·4 c.c.
	C	Guinea-pig infected with *T. evansi*. The citrated plasma containing 350,000 trypanosomes per c.mm.	24·7 c.c.	22·6 c.c.	0·2 c.c.	1·7 c.c.		
8	B	Normal guinea-pig	33·4 c.c.	24·0 c.c.	7·7 c.c.	1·8 c.c.	7·1 c.c.	0·1 c.c.
	C	Guinea-pig infected with *T. evansi*. The citrated plasma containing 450,000 trypanosomes per c.mm.	26·4 c.c.	24·1 c.c.	0·6 c.c.	1·8 c.c.		
9	B	Normal guinea-pig	37·3 c.c.	27·2 c.c.	8·2 c.c.	1·9 c.c.	7·1 c.c.	− 1·9c.c.
	C	Guinea-pig infected with *T. evansi*. The citrated plasma containing 395,000 trypanosomes per c.mm.	27·3 c.c.	25·3 c.c.	1·1 c.c.	1·9 c.c.		
10	B	Normal rat	31·1 c.c.	19·1 c.c.	10·0 c.c.	2·0 c.c.	5·1 c.c.	3·5 c.c.
	C.	Rat infected with *T. dimorphon*. The citrated plasma containing 150,000 trypanosomes per c.mm.	29·3 c.c.	22·6 c.c.	4·9 c.c.	1·9 c.c.		
11	B	Normal rat	24·6 c.c.	13·8 c.c.	8·7 c.c.	2·1 c.c.	6·9 c.c.	2·0 c.c.
	C	Rat infected with *T. evansi*. The citrated plasma containing 250,000 trypanosomes per c.mm.	19·6 c.c.	15·8 c.c.	1·8 c.c.	1·9 c.c.		

Table 4. Analysis of gases obtained from defibrinated blood and from mixtures of this with plasma of infected animals before and after incubation at 37° C. for 1 hour in the absence of air.

No. of experiment		Composition of Blood Mixture		Analysis of Gases obtained from Blood Mixture before and after Heating for 1 hour at 37° C. in the Absence of Air					
		Amount of defibrinated blood of normal rabbit	Amount of citrated plasma of infected animal consisting of 2 parts plasma and 1 part citrated saline solution	Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
1	A	3 c.c.	—	0·83 c.c.	0·26 c.c.	0·52 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. brucei* containing 37,500 trypanosomes per c.mm. Before incubation	1·16 c.c.	0·61 c.c.	0·48 c.c.	0·07 c.c.		
	C	,,	As B after incubation	1·07 c.c.	0·65 c.c.	0·35 c.c.	0·07 c.c.	0·13 c.c.	0·04 c.c.
2	A	3 c.c.	—	0·91 c.c.	0·43 c.c.	0·39 c.c.	0·08 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 100,000 trypanosomes per c.mm. Before incubation	1·13 c.c.	0·64 c.c.	0·36 c.c.	0·12 c.c.		
	C	,,	As B after incubation	0·82 c.c.	0·72 c.c.	0·03 c.c.	0·07 c.c.	0·33 c.c.	0·08 c.c.
3	A	3 c.c.	—	1·04 c.c.	0·42 c.c.	0·52 c.c.	0·09 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 35,000 trypanosomes per c.mm. Before incubation	1·27 c.c.	0·66 c.c.	0·52 c.c.	0·09 c.c.		
	C	,,	As B after incubation	1·17 c.c.	0·68 c.c.	0·39 c.c.	0·10 c.c.	0·13 c.c.	0·02 c.c.
4	A	3 c.c.	—	1·24 c.c.	0·68 c.c.	0·47 c.c.	0·09 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 287,000 trypanosomes per c.mm. Before incubation	1·27 c.c.	0·77 c.c.	0·41 c.c.	0·08 c.c.		
	C	,,	As B after incubation	1·11 c.c.	0·99 c.c.	0·05 c.c.	0·07 c.c.	0·36 c.c.	0·22 c.c.

TABLE 5. Data given in Table 4 re-calculated in volumes per cent.

No. of experiment		100 c.c. of mixture consisting of 2 parts defibrinated blood of normal rabbit and 1 part of citrated plasma of infected guinea-pig	Analysis of Gases obtained from Blood Mixture before and after Heating at 37° C. for 1 Hour in Absence of Air					
			Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
		Guinea-pig infected with *T. brucei*. The citrated plasma contained 37,500 trypanosomes per c.mm.						
1	B	Before incubation	25·8 c.c.	13·6 c.c.	10·7 c.c.	1·5 c.c.	2·9 c.c.	0·8 c.c.
	C	After incubation	23·8 c.c.	14·4 c.c.	7·8 c.c.	1·5 c.c.		
		Guinea-pig infected with *T. evansi*. The citrated plasma contained 100,000 trypanosomes per c.mm.						
2	B	Before incubation	25·1 c.c	14·2 c c.	8·0 c.c.	2·7 c.c.	7·3 c.c.	1·8 c.c.
	C	After incubation	18·2 c.c.	16·0 c.c.	0·7 c.c.	1·6 c.c.		
		Guinea-pig infected with *T. brucei*. The citrated plasma contained 35,000 trypanosomes per c.mm.						
3	B	Before incubation	28·2 c.c.	14·7 c.c.	11·6 c.c.	2·0 c.c.	2·9 c.c.	0·4 c.c.
	C	After incubation	26·0 c.c.	15·1 c.c.	8·7 c.c.	2·2 c.c.		
		Guinea-pig infected with *T. evansi*. The citrated plasma contained 287,000 trypanosomes per c.mm.						
4	B	Before incubation	28·2 c.c.	17·1 c.c.	9·1 c.c.	1·8 c.c.	8·0 c.c.	4·9 c.c.
	C	After incubation	24·7 c.c.	22·0 c.c.	1·1 c.c.	1·5 c.c.		

Apparatus.—The apparatus employed for this purpose was that illustrated by Figure I. The portion of the apparatus between Stop-cocks 1 and 2 was first filled with mercury. Through the two-way Cock 2 half a cubic centimetre of phosphoric acid (1 per cent. solution) was introduced into the vacuum Bulbs B to facilitate the removal of carbon dioxide. Cock 2 was now closed and a vacuum created in Bulbs B. The mixture of defibrinated blood and citrated plasma was then introduced by means of the receiver into Bulb A which communicated with the outside through Cock 1. Subsequently mercury was poured into the receiver and allowed to flow into the bulb until the blood just reached Cock 1, which was then turned so that the blood was now in contact with mercury both above and below. After allowing the mixture to react in Bulb A at 37° C. for one hour, Cock 2 was cautiously opened and the blood allowed to pass slowly into the vacuum bulbs. Cock 2 was then closed and the blood exhausted and the gases collected in Tube 1.

After complete exhaustion of the blood the gas collected in Tube 1 was measured and analysed by means of the apparatus indicated by figure 2.

Technique.—About 12 c.c. of blood was withdrawn from the external jugular vein of a normal rabbit and defibrinated by shaking with a few glass beads in a bottle. The blood was then filtered through gauze and placed in the ice-chest over night. The following morning it was thoroughly stirred and, after determining the percentage of haemoglobin present (in terms of human wet red cells), three samples each of 3 c.c. withdrawn and allowed to stand at room temperature, 10-12° C.

One of the three samples of defibrinated blood was then introduced into the apparatus in the manner described, and after warming to 37° C. for one hour in Bulb A was exhausted in the vacuum Bulbs B and the gases obtained measured and analysed.

To the second sample of defibrinated blood was added a definite volume of citrated plasma from an infected rat or guinea-pig containing numerous trypanosomes in suspension. (The number of trypanosomes per cubic millimetre in the citrated plasma was estimated by means of a Thoma Zeiss haemocytometer.) The mixture of blood and citrated plasma was then heated at 37° C. for

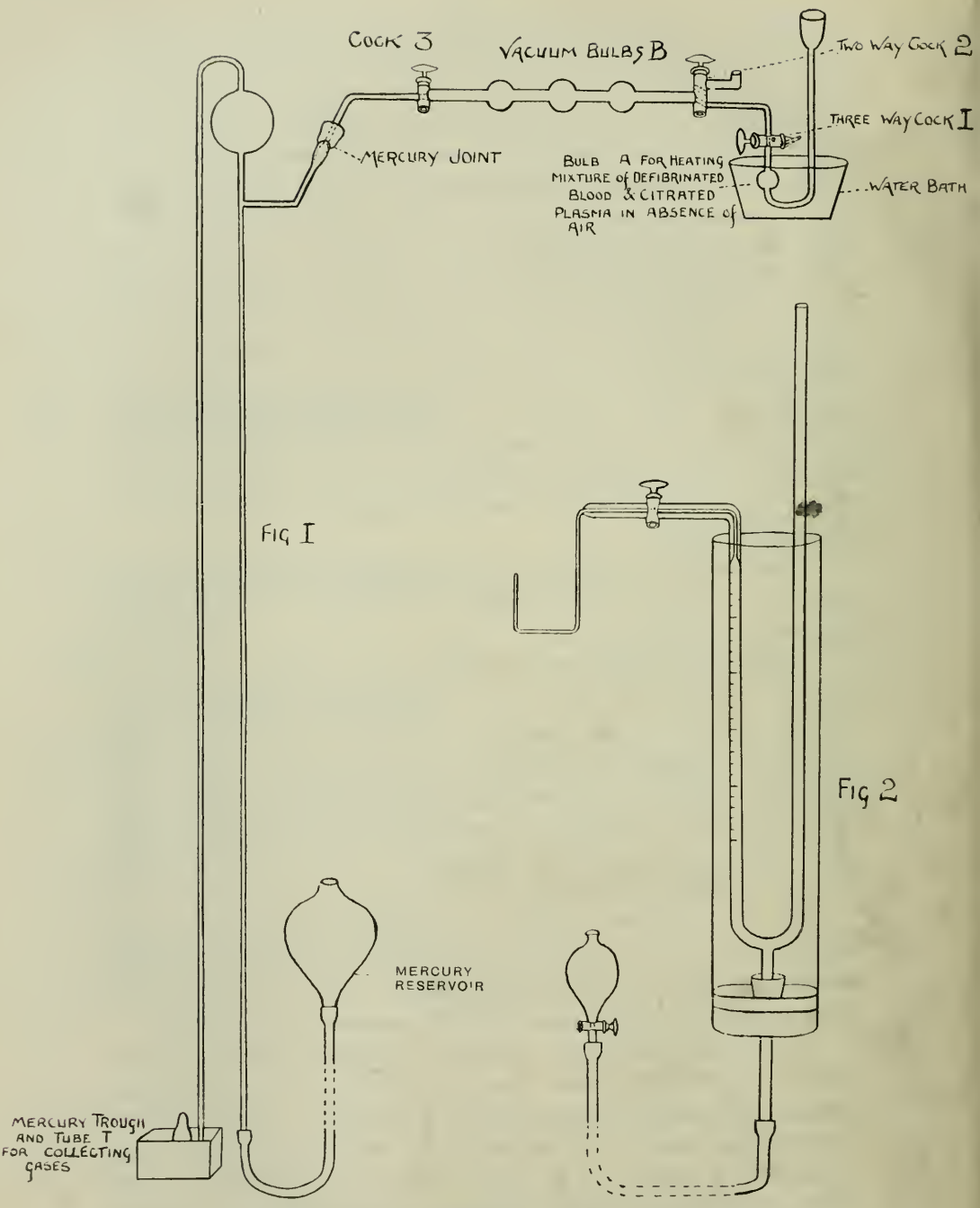

Fig. 1. Apparatus employed for incubating the suspension of trypanosomes and defibrinated blood in the absence of air, and for the subsequent removal of the gases contained in the blood mixture.

Fig. 2. Apparatus employed for measuring and analysing the gases.

one hour in Bulb A and the gases it contained subsequently determined.

A like volume of citrated plasma (diluted to the same degree as in the previous case) of a normal rat or guinea-pig was added to the third sample, which was then treated in the same manner as the other two cases.

The results obtained in these experiments are set forth in Table 2. It will be observed that in every case the action of the trypanosomes resulted in practically complete disappearance of oxygen from the gases subsequently exhausted from the mixture of defibrinated blood and infected plasma. A second point of interest is that the amount of carbon dioxide was not increased in a degree corresponding to the diminution of oxygen.

In Table 3 the results given in Table 2 are re-calculated in volumes per cent.; or, in other words, the quantity of gas present in 100 c.c. of the mixture—defibrinated blood plasma—estimated.

In a second series of experiments the procedure was similar, except that instead of employing as controls the plasma of a normal animal that of the infected animal itself was used. Here, however, reaction was not permitted to proceed in Bulb A of the apparatus, but the blood and plasma were passed straight on into the vacuum bulbs and immediately exhausted.

As will be seen from Tables 4 and 5 the results obtained are similar to those of the first series of experiments.

Although actively motile trypanosomes cause so considerable a reduction of solutions of methylene blue and haemoglobin *in vitro*, yet the mere presence of numerous parasites in the blood of the living organism is of itself insufficient to give rise to a purple condition of the blood. The blood of rats swarming with parasites is generally of the normal red colour. Under these circumstances the oxygenation of the haemoglobin occurring in the lungs is sufficient to counterbalance the reduction resulting from the action of the parasites.

As we have already mentioned, the purple appearance is most frequently to be observed in the blood of rabbits in a late stage of the disease. In these animals trypanosomes are usually absent from the peripheral blood or present in small numbers only. All animals presenting the phenomenon had, however, marked involvement of

the respiratory passages and the breathing was stertorous and laboured. The external nares was often almost completely obliterated by an oedematous and infiltrated condition of the skin and mucous membrane. Post mortem examination showed extensive thickening of the mucous membrane of the nose and pharynx, and sometimes even of the trachea and larger bronchi. Sections of the affected tissues usually revealed the presence of trypanosomes often in very considerable numbers.

SUMMARY

The blood of certain animals in the later stages of trypanosomal infections is frequently of a dark purple colour. This appearance results from deficient oxygenation of the haemoglobin.

Living trypanosomes cause marked reduction of solutions of methylene blue and also of those of oxyhaemoglobin.

The incubation, in the absence of air, of living trypanosomes in defibrinated blood of a normal animal causes considerable reduction or—if the parasites be numerous—total disappearance of the oxygen combined with the haemoglobin. A corresponding increase in the amount of carbon dioxide has not been found.

The mere presence of numerous parasites in the peripheral circulation is not, however, sufficient to account for the purple colour of the blood, since the blood of rats and guinea-pigs swarming with parasites is usually of the normal bright-red appearance.

The purple colour is most marked in the blood of rabbits in the later stages of the disease. Although the peripheral blood of these animals does not contain large numbers of parasites, yet the respiratory passages are found to be considerably involved. The nasal mucous membrane and that of the trachea and bronchial tubes is often extremely oedematous and infiltrated, and the respiration of the animal is stertorous and laboured. On microscopical examinations of sections of these tissues trypanosomes were often found in large numbers in the oedematous mucous and sub-mucous tissue.